METABOLIC STUDIES ON ANIMALS IN OXYGEN AT A SIMULATED ALTITUDE OF 26,000 FEET

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Introduction

One of the paramount questions to be answered with maximum haste is what parameters should be used to measure the effect of the flight environment on the health, safety, and efficiency of an astronaut. Since any adverse effects of a particular environment would be expected to influence metabolic activity, our experiments were designed to study the effect of environment on metabolic pools.

Methods

The environmental condition chosen for the experimental animals was 93% oxygen with an atmospheric pressure of 5.22 psi. Control animals were maintained under normal atmospheric conditions. Twenty-four male Holtzman rats were divided into four groups, with each group having six rats with an average weight of 360 grams. Two of these groups served as experimental animals and were housed in steel environmental chambers with glass windows. The other two groups served as control animals and were housed in identical chambers except these were constructed of wood. The gaseous environment in the experimental chambers was provided by passing oxygen into the chamber through a Collins gasometer, which served as a reservoir and provided water-saturation of the oxygen. A Welch Duo-seal vacuum pump with needle valves was used to maintain the desired atmospheric pressure. The flow rate of the water-saturated oxygen was maintained at a

constant rate of 350 ml per minute. The control chambers had air pulled through them at the same rate as oxygen was added to the experimental chambers. This was done by forming a slight vacuum with a water aspirator. All rats were exposed to these conditions for 8 hours each day. During the remaining 16 hours, they were kept in individual metal cages under normal atmospheric conditions.

All four chambers were maintained at approximately 26° C and all contained soda lime to trap CO_2 and silica gel to absorb water. Food and water were given ad libitum. To reduce the chance of chronic or sub-clinical diseases, especially respiratory diseases, Tylosin tartrate was added to the drinking water at a concentration of 500 µg/gal. The rats were allowed free movement in the chamber; that is, they were not placed into individual restraining cages.

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Results and Discussion

After exposure to this environment for thirty-four days, eighteen of the animals were sacrificed. Comparing average body weights and the organ weights, which are expressed as per cents of body weights, (Table 1), it is obvious that there were no significant differences in these parameters. Further, the experimental animals looked normal.

Lipid was extracted from the liver, heart, kidney, brain, and muscle. The weight of this lipid expressed as the per cent of organ weight is shown in Table 2. We were unable to demonstrate any significant change except perhaps in the heart lipid, but even this was significant only at the 5% level. However, the trend was in the direction of higher lipid content in the experimental animals.

The serum proteins were analyzed by ultracentrifugation. Using equivalent amounts of serum from each of the experimental animals as a pool to compare

a similar pool of controls, a preparative run to separate lipoproteins was made for twenty-four hours at 20° C at 104,000 x g in a KBr - NaCl solution, the final density of which was 1.21. The experimental animals quantitatively contained one half the amount of lipoprotein that the control animals contained. Additionally, an analytical run was made of the proteins per se. This analysis showed a marked reduction in the 19-S fraction.

Based upon a pilot experiment, we anticipated changes in metabolism even though no major changes in body composition were observed except for the ones outlined above. Therefore, after the animals were maintained in the chambers for eight hours per day for six days, a series of injections was begun using sodium acetate-2-C^{14} at the rate of 60 uc per kg of body weight. The injections were made intraperitoneally. After exposure to the environmental conditions for an additional twenty-eight days, all the animals were sacrificed. Pairs of animals (one experimental and one control) had been injected at one-half hour, 8 hours, and 1, 4, 9, 14, 21, and 28 days prior to sacrifice. All injections were made at 7:30 am.

An aliquot of the CO_2 trapped during the first eight hours after injection with radioacetate was taken from both the control and experimental chambers and analyzed for radioactive CO_2. The radioactive CO_2 expired by the experimental animals is expressed as a per cent of the control animals in Table 3. The expiration of labeled CO_2 by the control animals was fairly constant. The experimental animals apparently were metabolizing at a lower rate than the control animals and as the experiment progressed the experimental animals apparently did not adapt to the environment but metabolized at even a slower rate.

Table 4 shows a typical series of results over a three-day period after injection. During the first eight-hour period post-injection, the control animal

expired more of the radioactivity as CO_2 than the experimental animal. The second day this trend was reversed, but the third day after injection the control was again expiring more radioactive CO_2. These results were typical for the three days post-injection in all of the experiments during the whole injection series. Several interpretations are possible. Perhaps the radio-CO_2 expired on the day of injection represents the catabolism of acetic acid per se. If this is true, the catabolic rate for radio-acetate is greater in the control animals. The CO_2 expired the day after injection may represent an interim time in the metabolism of acetate; that is, in the control animals acetate has for the most part either been catabolized to CO_2 or incorporated into the body's metabolic pools. On the second day post injection, perhaps the increase in the expired radio-CO_2 by the control animals is a result of catabolism of the body's metabolic pools. If this interpretation is true, then the data would suggest again that the experimental animals have a lower catabolic rate. The alternative is that the experimental animals are converting the acetate into metabolic products at a greater rate than the experimental animals. If this be the case, then there would be less radio-acetate for the experimental animals to catabolize to CO_2. To differentiate these two possibilities, the radioactivity in the tissue pools was determined.

Each of the five tissues indicated above (liver, heart, kidney, brain, and skeletal muscle) were extracted twice with a chloroform:methanol:water system (1:1:0.9). The incorporation of radioacetate into lipids of brain and muscle was extremely low. Thus we were unable to demonstrate any difference. However in the liver, the incorporation of radioacetate was manyfold higher in the control (Figure 1), suggesting that lipid synthesis was reduced in the experimental animals.

A similar plot in the case of heart lipid (Figure 2) indicates that a similar reduction of lipid synthesis occurred. The earlier weight data showing that there is more lipid in the heart of experimental animals therefore suggest that catabolism is also reduced. The data on kidney lipid (Figure 3) suggests that, not only is there a decrease in the rate of synthesis, but also a reduction of the catabolic rate as well.

We wondered what types of lipid were most effected. We therefore pooled equivalent amounts of the liver lipids of the experimental animals injected half-hour, eight-hour, and one-day prior to sacrifice and compared these lipids with the corresponding ones obtained from the control animals. These pools were chromatographed on silicic acid columns and eluted by the scheme of Barron and Hanahan into the fractions indicated in Table 5. The first two columns of figures show the weight of the fractions expressed as a per cent of the total lipid and indicate the major alteration was a total decrease in the hydrocarbon and sterol ester fractions with a concomitant increase in the triglyceride and unesterified fatty acid (UFA). The next two columns show the incorporation of radioacetate into the corresponding fractions. These indicate that there was a tremendous decrease in total fat synthesis particularly in the triglyceride and UFA, free sterols, and phospholipid fractions. However the data presented in this form do not clearly indicate whether this is a general decrease or a rather specific effect. Table 6 shows the specific activity of these lipids expressed as dpm per mg. The major alteration here is observed in the Triglyceride and UFA fraction as well as the total lipids. The general trend in all of the last four fractions is toward a decrease in specific activity. The second column shows the specific activity expressed as a per cent activity/unit weight. This gives us an indication of where the acetate was incorporated. These data

indicate that the more dramatic alteration occurred in the triglyceride + UFA fraction, suggesting that fatty acid synthesis is drastically reduced. (Approximately 95% of the weight of a triglyceride is due to the fatty acids.) Although anabolism was impressively reduced, catabolism was probably even more reduced. Thus the apparent result was an increase in total tissue lipid.

Since these data suggest a decrease in fatty acid synthesis, the natural question would be, "Is this a general decrease in fatty acid synthesis, or are certain fatty acids more effected than others?" A gas-liquid chromatogram of the fatty acids in the liver of the control animals (Figure 4) shows the relative amount of each fatty acid present. The fat is the same as that used in the column chromatography analysis. Note the specific activity (% of radioactivity/mole %) under each of the major peaks. Note also the presence of a C_{16} fatty acid which has a greater retention time than palmitoleic acid but less than heptadecanoic acid. In the chromatogram of liver fatty acids of the experimental animals, (Figure 5), note that this sixteen carbon peak is almost absent and that one of the C_{14} peaks (not 14:0) is dramatically reduced. Otherwise the quantitative aspects are not particularly altered. We are not at this time sure what the C_{16} acid is. Additionally, the specific activity of the oleic acid is greatly reduced. It is therefore possible that there is some alteration in the rate of synthesis of the unsaturated fatty acids.

Reflecting on our ultracentrifuge work, a decrease in lipid anabolism and catabolism would be expected to result in a decrease in lipid transport so one might see a decrease in serum lipoprotein, especially the high density lipoprotein associated with the chylomicron. Thus both sets of data support the same thesis.

In order to get some indication of carbohydrate pools, the lipid extracted homogenate was made 5% with respect to trichloroacetic acid (TCA) to precipitate

the protein. The radioactivity in the TCA-soluble material is shown on Table 7. Similar to our CO_2 data, the control animals initially show a greater incorporation of acetate. However, after twenty-four hours the trend is reversed. If one compared the control animals at eight and twenty-four hours, one sees an active catabolism. This is not readily apparent when you compare the experimental animals at eight and twenty-four hours. It should be observed that the values for the kidney in both instances are higher than the corresponding control.

The TCA-insoluble portion was washed with 20% TCA, solubilized at pH 8.2 and the protein concentration determined spectrophotometrically at 260-280 mμ. The radioactivity incorporation was so low that no appreciable activity was observed in either the muscle or the brain. However, Table 8 shows the specific activity in the TCA-insoluble fraction of the liver, heart, and kidney. With respect to the liver, again the experimental animals show a slower rate of incorporation. No appreciable difference was observed in the heart, although activity was quite low. In the kidney, similar to the TCA-soluble fraction, the specific activity is constantly higher, suggesting, if anything, an increase in the metabolic activity of the kidney. We did not determine whether acidosis existed in the experimental animals; but if this occurred, then the kidney may very well work harder than the kidney of the control animals. It is therefore plausible that the metabolic activity of this tissue may differ from the other tissues of the body.

The proteins in the serum were also analyzed by paper electrophoresis using a veronal buffer (diethyl barbituric acid and its sodium salt) (0.075 μ, pH 8.6). The strips were stained with bromphenol blue and quantitated in an analytrol. No consistent differences were observed quantitatively in the various fractions between the

experimental and control animals. However, the specific activities after eight and twenty-four hours (Table 9) show once again that in the eight-hour animals the control tends to be a little higher. After twenty-four hours the experimental animals are consistently higher. Again compare the experimental animals at eight and twenty-four hours. Less reduction in specific activity is observed in the experimental animals, suggesting a reduction in catabolism.

Conclusions

We must emphasize at this point that these data are the result of one full experiment and one pilot experiment. We intend to challenge these results in a replicate experiment. However in the next experiment we intend to expose the animal to the environment continuously for twenty-eight days. The impressive points demonstrated in the experiment reported here are that metabolism in general reduced by at least 10% and perhaps even more, that metabolically speaking the animals do not appear to be adapting to the experimental environment, and that the metabolic alterations observed could not be repaired in the sixteen hours per day that the animals were outside the chamber. These parameters, therefore, represent those most sensitive in an irreversible manner to the "space capsule" environment.

Perhaps the utilization of the lipoprotein measurement in serum should be further investigated as a possible clinical indicator of metabolic damage in an astronaut.

Finally it must be emphasized that (1) these metabolic alterations were observed in spite of the fact that there were no differences in body weight nor gross

differences in body composition; (2) we do not know whether these errors in metabolism can easily be repaired after cessation of exposure to the experimental environment; and (3) we do not know if these errors in metabolism are due to the effects of O_2, the absence of N_2, or barometric pressure. The relationship of these data to general tissue catabolism as measured by the activity of lysozomal enzymes and the oxidation of metabolites, enzymes and cofactors are currently under investigation in our laboratory.

TABLE 1

BODY AND ORGAN WEIGHTS

	Control	Experimental
Avg. Body Weight (gms)*	405	403
% of Body Weight		
Liver	3.0	3.1
Kidney	0.7	0.7
Brain	0.3	0.4
Heart	0.2	0.2

* At the time of sacrifice

TABLE 2

LIPID AS % OF ORGAN WEIGHT

	Control	Experimental
Liver	1.6	1.8
Heart	1.9	3.5*
Kidney	3.1	3.6
Brain	6.0	5.7
Muscle	5.2	8.1

* Significantly greater than the control (P=0.05)

TABLE 3

$C^{14}O_2$ EXPIRATION BY EXPERIMENTAL ANIMALS IN FIRST 8 HOURS POST-INJECTION

(expressed as % of paired control animal)

Day*	% of Control
8	90
20	79
28	77

* Day of injection in the 28 day experiment

TABLE 4

$C^{14}O_2$ EXPIRATION

(for the 1st 8-hour period of each day indicated)

DAY*		dpm
20	Control	186,390
	Experimental	108,838
21	Control	564
	Experimental	1,714
22	Control	1,628
	Experimental	1,071

* Day of the 28 day experiment

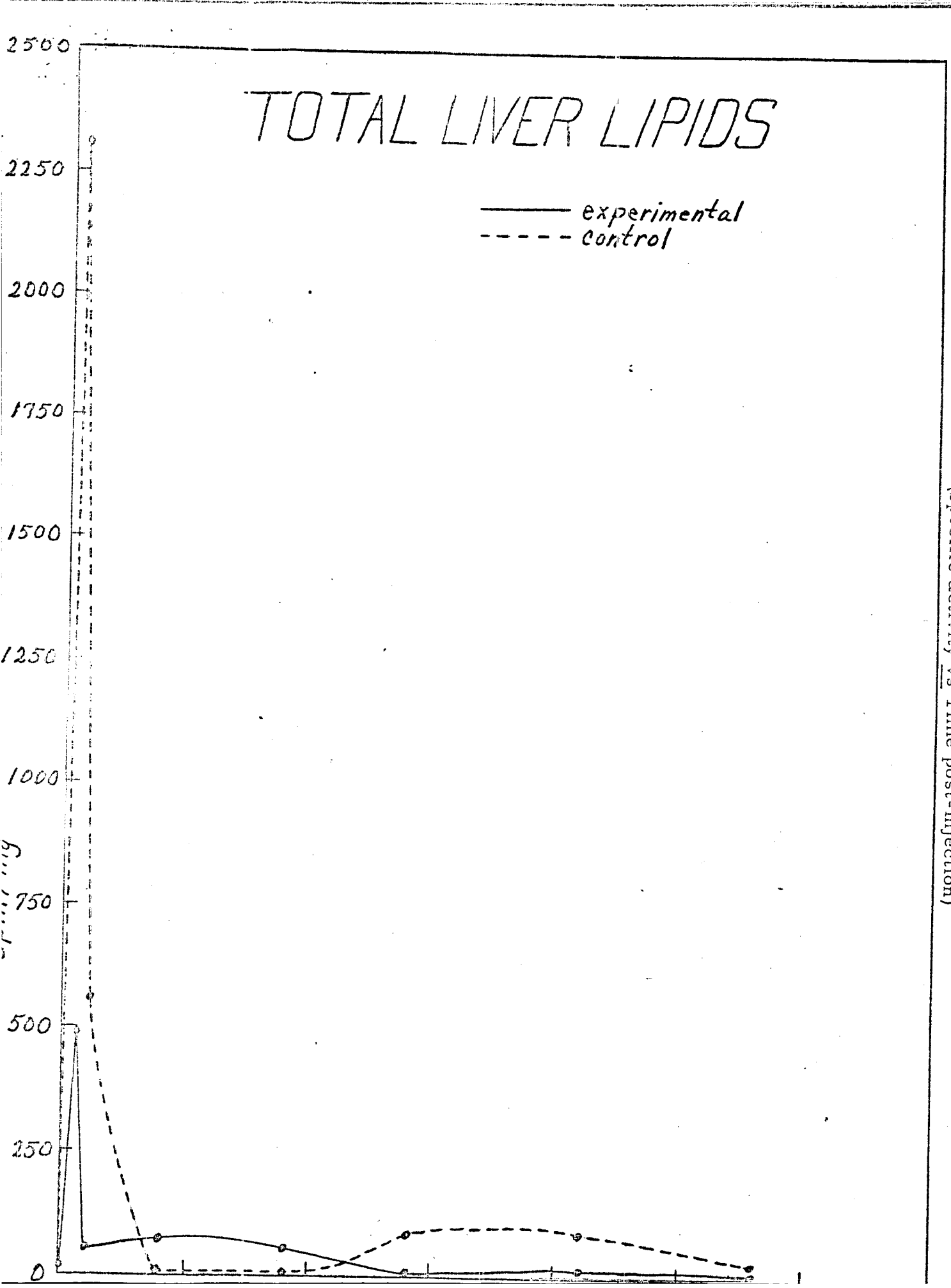


FIGURE 1. Total Liver Lipids
(Specific activity vs Time post-injection)

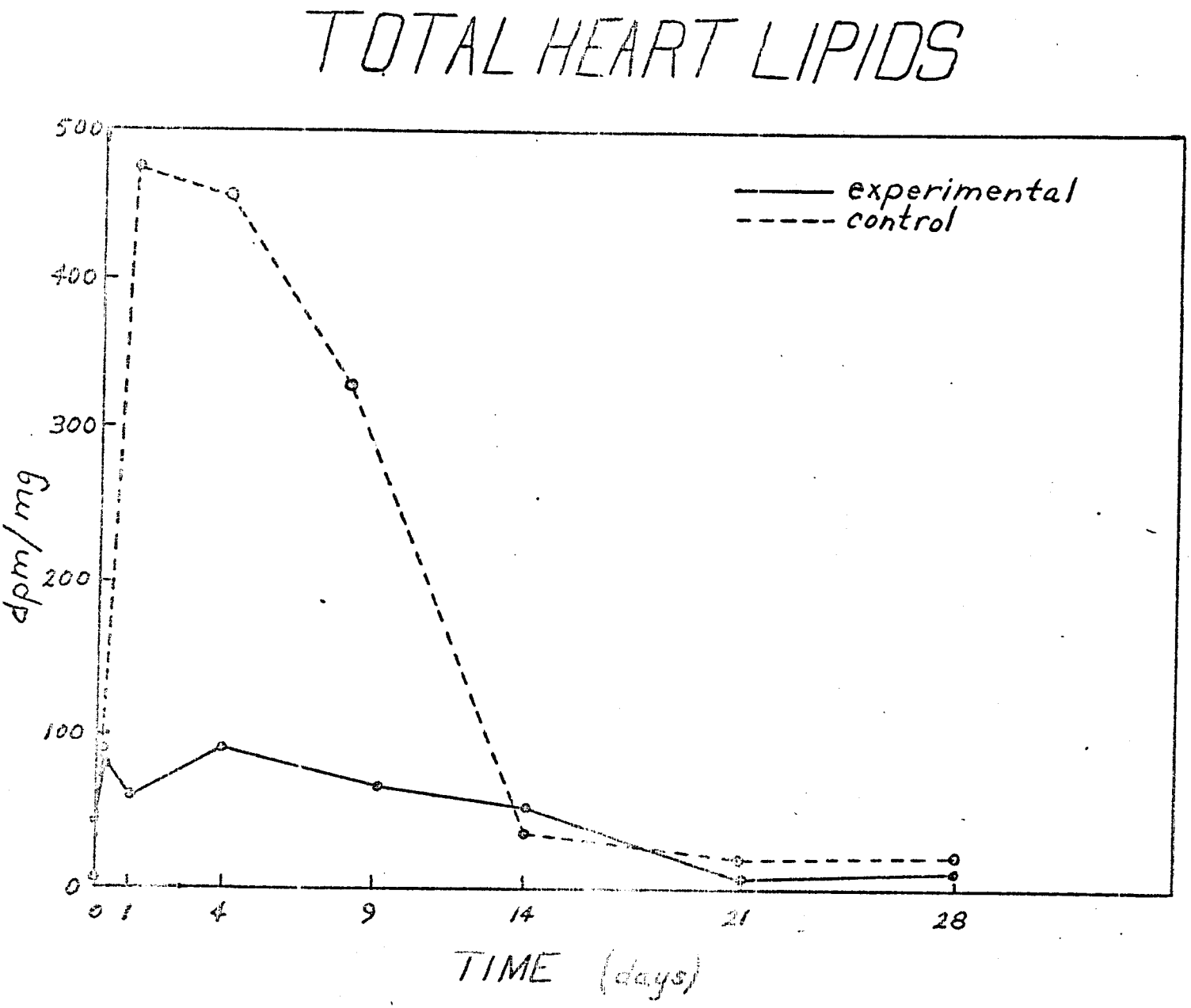


FIGURE 2. Total Heart Lipids
(Specific activity vs time post-injection)

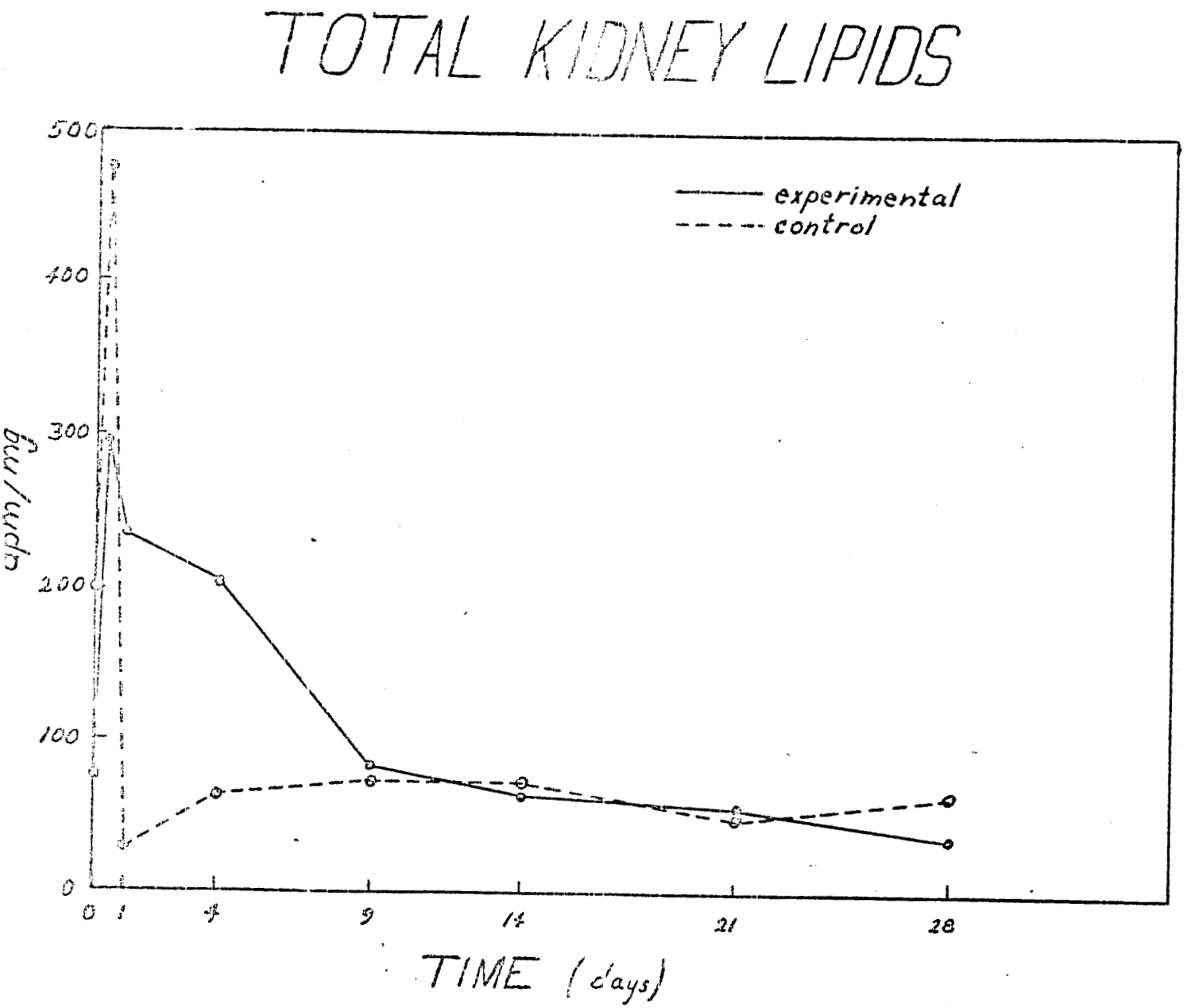
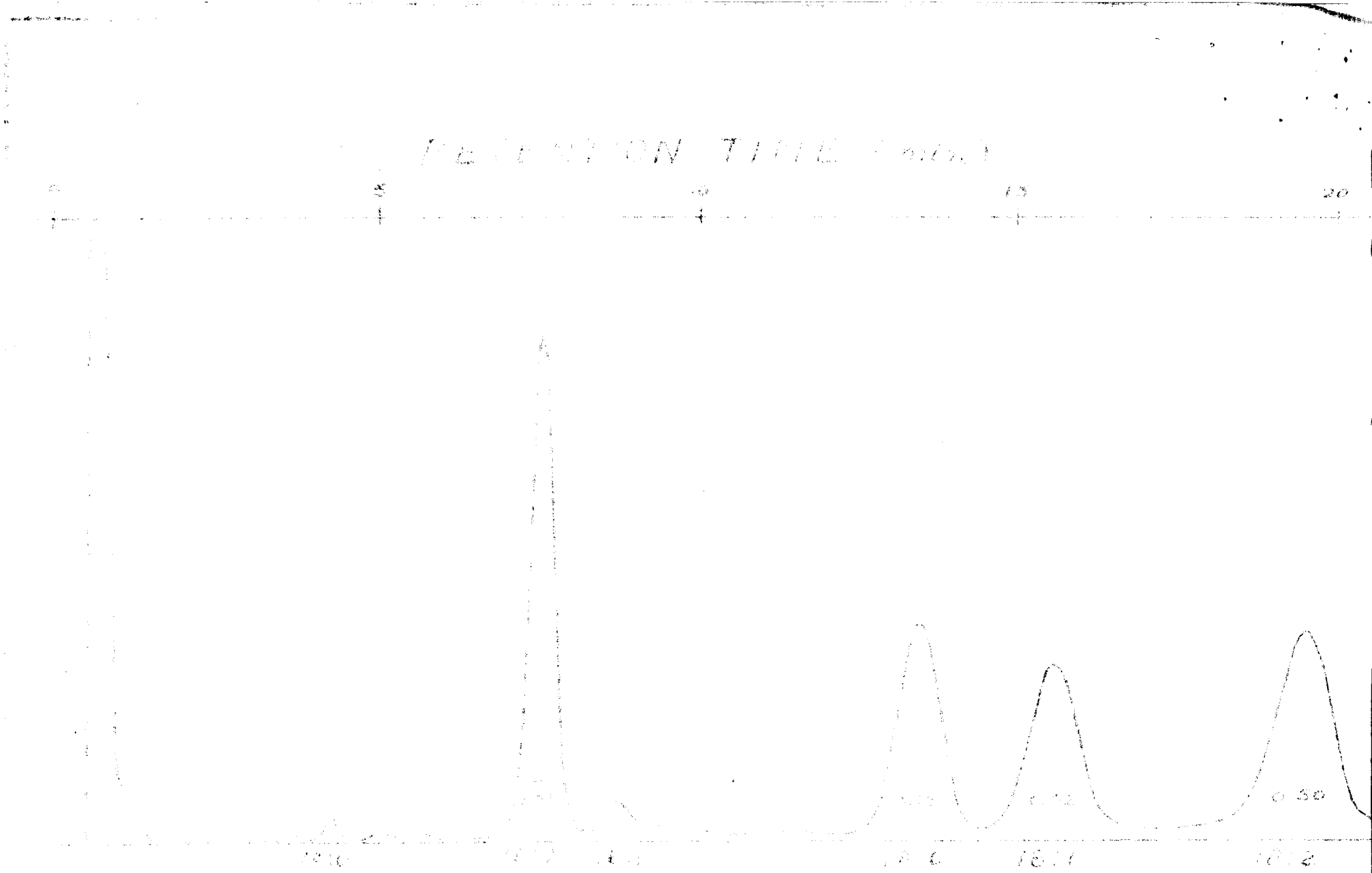


FIGURE 3. Total Kidney Lipids
(Specific activity vs time post-injection)

TABLE 7

^{14}C INCORPORATION INTO TRICHLOROACETIC ACID INSOLUBLE FRACTION
(d.p.m.)

	8 hrs post-injection		24 hrs post-injection	
	Control	Experimental	Control	Experimental
Liver	765	614	609	214
Heart	136	49	41	37
Kidney	266	368	86	458
Brain	123	97	29	95
Muscle	127	241	106	119



LIVER RATIO RATES IN
EXPERIMENTAL ANIMALS

TABLE 9

SPECIFIC ACTIVITY OF SERUM PROTEINS
(dpm/mg)

	8 hrs post-injection		24 hrs post-injection	
	Control	Experimental	Control	Experimental
Albumin	66	46	30	41
α_1 Globulin	337	310	227	266
α_2 Globulin	774	742	56	102
β Globulin	308	220	103	111
γ Globulin	148	158	82	139

TABLE 5

[illegible]
(ppm w/w basis)

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	307	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	73	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	93	[illegible]	[illegible]	[illegible]

[illegible]

TABLE 5

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE

	Farming			
	Control	Experimental		
[illegible]	91	84	0.05	0.54
[illegible]	1	1	0.000	0.1
[illegible]	2239	260	5.47	1.15
[illegible]	133	112	0.54	2.1
[illegible]	119	208	0.4	1.1
[illegible]	97	137	0.19	1.31
[illegible]	127	171		